Journal of the American Mosquito Control Association, 10(3):363–373, 1994

INTEGRATED MANAGEMENT OF WASTE TIRE MOSQUITOES UTILIZING *MESOCYCLOPS LONGISETUS* (COPEPODA: CYCLOPIDAE), *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS, BACILLUS SPHAERICUS,* AND METHOPRENE

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ABSTRACT. This study evaluated the compatibility and efficacy of using a predatory copepod, *Mesocyclops longisetus* in concert with 3 "biorational" compounds for mosquito control in waste tires. The toxicity of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), *Bacillus sphaericus,* and methoprene to *Mesocyclops longisetus* was assessed in the laboratory using concentrations 10 times the maximum labeled or suggested rate and based on a water depth of 7.6 cm. Microbials were tested using mature copepods exposed for durations of 24, 48, and 72 h. Methoprene bioassays consisted of individually exposing newly hatched copepods (i.e., nauplius larvae) and monitoring their development to maturity. The toxicity tests indicated *B.t.i., B. sphaericus,* and methoprene were not deleterious to copepods at concentrations exceeding those expected in the field. Copepods exposed to methoprene matured normally, and when mated, 50% developed egg sacs. A 5-month field test, integrating the copepod and *B.t.i., B. sphaericus,* and methoprene provided better mosquito reduction together than either copepods or control agents alone. When copepods were combined with *B.t.i.* or methoprene, overall reduction of 3rd- and 4th-instar larvae during the 5-month interval was equal to or greater than 90%. *Bacillus thuringiensis* var. *israelensis* alone temporarily produced a high degree of larval reduction (up to 100%), however reapplications were necessary to maintain that level of control. Of all the treatments, *B. sphaericus* alone produced the lowest degree of mosquito suppression due to lack of toxicity to *Aedes albopictus,* the predominant species during the study. It is recommended that mosquito control managers consider integrating *M. longisetus* and *B.t.i.* or methoprene against mosquitoes in waste tires.

INTRODUCTION

Certain cyclopoid copepods are predators of mosquito larvae (Bonnett and Mukaida 1957, Riviere and Thirel 1981, Marten 1984) and some have been studied as potential biological control agents for mosquitoes (Suarez et al. 1984; Riviere et al. 1987; Marten 1990a, 1990b). In Tahiti, the copepod *Mesocyclops leuckarti pilosa* (Daday) was reported to eliminate *Aedes aegypti* (Linn.) and *Aedes polynesiensis* Marks larvae from containers but had negligible impact on *Culex quinquefasciatus* Say larvae (Riviere and Thirel 1981). This was attributed to this copepod's benthic distribution, which made it a more efficacious predator of bottom-dwelling mosquitoes. Recent studies have focused on evaluating cyclopoid copepods for control of *Aedes albopictus* (Skuse), after this mosquito's rapid colonization in southeastern United States (Marten 1990a, 1990b). Marten (1990b) found the copepod *Macrocyclops albidus* (Jurine) to be a promising biological control agent for *Ae. albopictus* in tire piles. In a wooded area of New Orleans, he found that this predator completely eliminated mosquito larvae within 2 months postinoculation, and continued to do so for 2 months. The reason for the initial delay in larval reduction was 2-fold: due to time needed for the copepods to increase their population and exert predation pressure on the larvae, but also because the copepods were only capturing early instar larvae. If later instars were present during the initial inoculation, these would likely escape predation and later emerge as adults (Marten 1990b). The same phenomenon was reported for tadpole shrimp and asynchronously developing *Culex* larvae (Tietze and Mulla 1989) and for *Toxorhynchites rutilus rutilus* (Coq.) (Schreiber and Hunter 1993). The problem of delayed effectiveness may be corrected by either starting with higher copepod inoculation rates (Marten 1990b) or by integrating chemical/microbial control with the predatory agent (Riviere et al. 1987). Theoretically, integrated control would not only reduce initial *Aedes* and *Culex* populations, but also confer long-lasting control via the biological control agent. If copepods are tolerant to other control agents, they may be applied in unison, where the first week(s) of mosquito reduction is largely due to the immediate effects of the control agent (e.g., *Bacillus thuringiensis* var. *israelensis,* etc.) and the subsequent weeks of reduction are due to copepod predation upon early instar larvae.

Bacillus thuringiensis var. *israelensis* (*B.t.i.*) has been found to be nontoxic to important nontargets, even to chemically sensitive taxa such as mayflies (Mulla et al. 1982). *Bacillus sphaericus* Neide did not affect field populations of copepods, amphipods, ostracods, and cladocerans when applied to a pond at a rate of 1×10^4 cells/ml (Miura et al. 1981). However, methoprene had mixed results. Miura and Takahashi (1973) found the insect growth regulator (IGR) ZR-515 at 28 g AI/ha negatively impacted copepods and cladocerans in acute toxicity tests conducted in the laboratory. In the field, however, no adverse effects were reported using a slow-release formulation of the same compound (Miura and Takahashi 1973). Early nauplii of *Apocyclops spartinus* (Ruber) exposed to methoprene had a 48-h LC_{50} of 0.8 ppm; that of late nauplii was 2.0 ppm (Bircher and Ruber 1988). Additional field studies by Schaefer et al. (1974) yielded no negative effects of methoprene on eucopepods, conchostracans, and cladocerans. Majori et al. (1977) tested Altosid (methoprene) against *Aedes detritus* (Haliday) and monitored nontarget populations. They found no adverse effect at 0.052 lb AI/acre to the copepod *Cyclops* sp., as population trends in treated plots closely followed that of controls.

Our study evaluated the effects of integrating *Mesocyclops longisetus* (Thiebaud) with 3 "biorational" agents (*B.t.i., B. sphaericus,* and methoprene) against waste tire mosquitoes. Goals of this study were 2-fold: 1) assess compatibility of the copepods and the larvicides at concentrations exceeding operational rates (10×), and 2) evaluate initial and long-term performance of these combinations as an integrated management strategy against wild mosquito populations in waste tires.

MATERIALS AND METHODS

Acute toxicity tests were conducted at the John A. Mulrennan, Sr. Research Laboratory (JAMSRL). Bioassays with microbials were run using 10 adult *M. longisetus* per 500 ml well water in a beaker immersed in a temperature-controlled water bath (27°C). The *B.t.i.* treatments were formulated from Teknar HP-D (Zoecon Corp., Dallas, TX) at 10× the suggested rate of 1.17 liter/ha (calculated for a depth of 7.62 cm of water) or 15,710 ppb. *Bacillus sphaericus* was prepared using Spherimos FC El 781 (No. FUN90D10A) supplied by Novo Nordisk (Denmark) based on the same application rates as *B.t.i.* Formulations of both *B.t.i.* and *B. sphaericus* stock suspensions were prepared in deionized water. Bioassays using *Bacillus* spp. were conducted in the following manner: treatments and controls consisted of 6 replicate beakers each; survival was assessed 24, 48, and 72 h posttreatment. The above test was conducted twice for each microbial agent.

Altosid Liquid Larvicide (0.0494 kg AI/liter or 0.43 lb AI/gal) (Zoecon Corp., Dallas, TX) was the source of methoprene in bioassays and tested at a concentration of 196.7 ng/ml (10× the concentration expected in water 7.62 cm deep). Methoprene bioassays necessitated exposing recently hatched nauplius larvae (i.e., less than 24 h) of *M. longisetus* to determine survival to maturity. Test populations of first stage nauplius larvae were collected by maintaining gravid copepods in small screened cages (mesh aperture = 290 μm), which permitted only the larvae to pass through the screen into the surrounding water. Thirty larvae were exposed to methoprene per 50 ml beaker; similar numbers of larvae were individually maintained as controls. Survival was assessed after 1, 2, 4, 7, and 9 days of exposure. It was necessary to feed the developing copepods *Paramecium* during the test due to the prolonged test period. This test was also extended to determine reproductive success of the mature female copepods. This was achieved by individually mating 6 treatment and 6 control female copepods with males of similar treatments after the 9-day test period and observing whether egg sacs were produced.

In field studies, 40 discarded tires were cleaned and radially sliced along one side to facilitate sampling and placed on the grounds of the Fannin Airport of Panama City, a wooded area consisting of slash pine (*Pinus elliottii* Englem.), live oak (*Quercus virginiana* Mill.), and magnolia (*Magnolia* sp.). Each "pile" consisted of 5 tires leaned against the trunk of a tree; this was done for 8 piles. Each pile was at least 30.5 m from the nearest tire pile. To enhance copepod survival during the first weeks of the study, infusions were created as an alternate food source by adding to each tire small amounts of leaf detritus, mostly pine needles.

Prior to inoculation with copepods, mosquito larval density within each tire was monitored using the following method. The entire contents of each tire were concentrated (mesh aperture = 290 μm), transferred to plastic containers, and transported to the laboratory for counting. To facilitate the counting process, acrylic trays were constructed to form lanes into which the sample was spread out. Using the laned acrylic trays, numbers of mosquitoes were enumerated by instar (1st and 2nds grouped together). Due to time constraints, a single mosquito density was determined for all species present per tire. Mosquito species were identified from each tire sam-

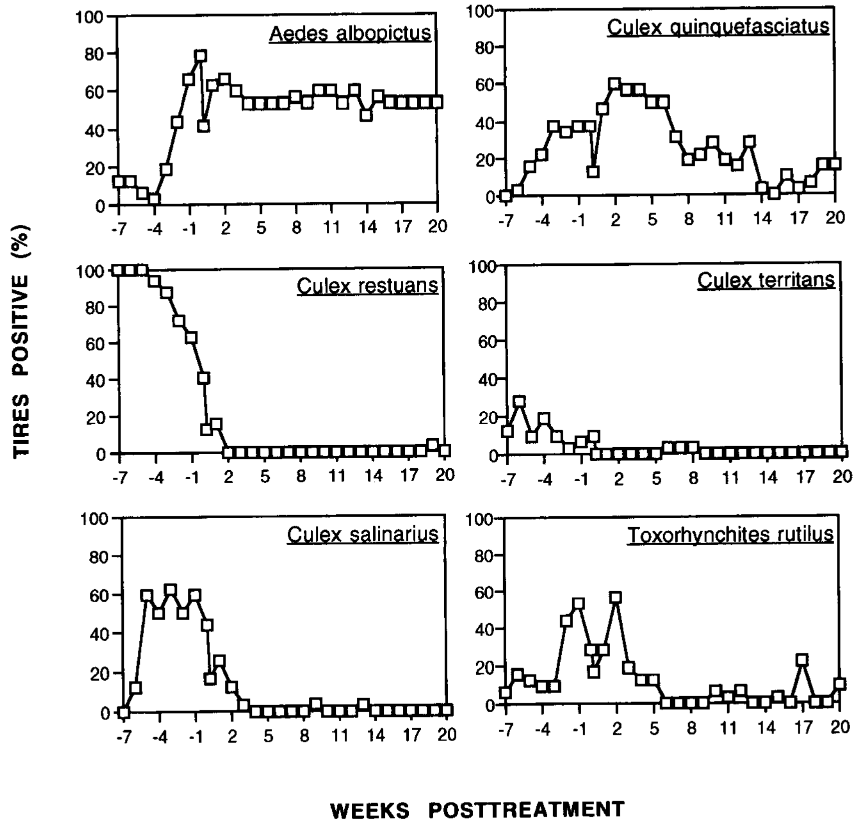

Fig. 1. Percent of tires positive for species of immature mosquitoes by week posttreatment averaged for all tires sampled during pre- and posttreatment intervals.

pled. Mosquito identifications were confirmed by specialists at JAMSRL and voucher specimens were maintained. The presence of immature midges, ostracods, and other tire-inhabiting organisms was noted. After counting, the samples were returned to their respective tires. Each tire was sampled once per week during the 6-wk pretreatment sampling interval.

Pretreatment mosquito populations were analyzed statistically using the Student-Newman-Keuls test (SAS Institute 1990) to determine whether densities differed between piles. The latter test was applied separately to analyze the dependent variables: 1) 1st and 2nd, 2) 3rd, and 3) 4th instars. To assess the abundance of different mosquito species, the percent tires positive for each pile was determined by crosstabulating pile by species (SAS Institute 1990).

Due to low precipitation during the pretreatment monitoring period, well water was added to 4 tires of each pile to maintain about 1-liter per tire; water was not added to the 5th tire of each pile to observe what would occur under natural drying conditions. On the 5th week of the pretreatment interval, all appropriate tires were inundated with about 4 liters of water and subsequently maintained at more than 1 liter per tire. The water volume of the 5th tire was a result of rainfall alone.

Copepod inoculations were made when *Ae. albopictus* populations were the predominant mosquito species within the tires. Prior to inoculation, each of the 8 treatments (*B.t.i.*, *B. sphaericus*, methoprene, *B.t.i.* and copepods, *B. sphaericus* and copepods, methoprene and copepods, copepods and controls) was randomly designated to a pile. *Mesocyclops* were obtained from a culture at JAMSRL and separated into groups of 100 by pipetting them off laned acrylic trays or microwell plates viewed under a dissection microscope. Copepods were immediately inoculated into the tires of the 4 randomly selected piles at a rate of 100 copepods per tire.

The microbials and methoprene were applied to the tires during the first week post-inoculation. It was necessary to make applications on consecutive days to facilitate the sampling process. Initial treatments using *B.t.i.*, *B. sphaericus*, and

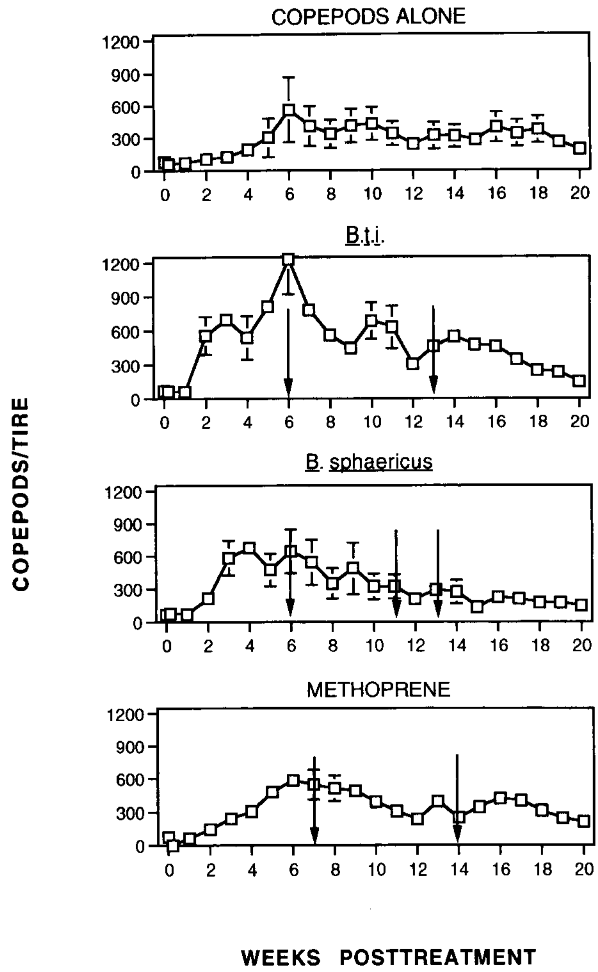

Fig. 2. Mean density of copepods in waste tire piles untreated or treated with *Bacillus thuringiensis* var. *israelensis*, *B. sphaericus*, and methoprene during 20-wk posttreatment interval. Arrows indicate time of reapplication of control agent.

methoprene were made on May 24, 26, and 27, 1993, respectively. Initial treatments and posttreatment sampling of each control agent were synchronized for tires with and without the copepods present. Samples were taken 2 days posttreatment and on weekly intervals thereafter for 20 wk. Treatment concentrations were 1,565 and 1,584 ppb for *B.t.i.* and *B. sphaericus*, respectively. Methoprene was applied to achieve a final concentration of 19.7 ppb.

During the posttreatment interval, mosquito populations were sampled as described for pretreatment monitoring. In addition, the absolute density of copepods (copepodid and adult stages) and water volume of the tires were determined for each tire. Minimum and maximum thermometers were used to monitor water temperatures each day tires were sampled. Weekly rainfall was monitored less than 1.6 km from the test site. Posttreatment sampling used separate collecting and counting equipment to preclude cross-contamination of the microbials/IGRs as well as unintended transfers of copepods between piles. Due to the abundance of *Tx. r. rutilus* in the tires, it was decided to remove these efficacious predators during weekly sampling to preclude increased variation in mosquito density due to their activities.

The efficacy of the different treatments was assessed by calculating percent reduction using the formula:

$$\%R_{ij} = [C_{ij} - T_{ij}/C_{ij}] \cdot 100$$

where R_{ij} is the reduction of instar/stage i during week j; C_{ij} is the average number of instar/stage i during week j in the control pile; and T_{ij} is the

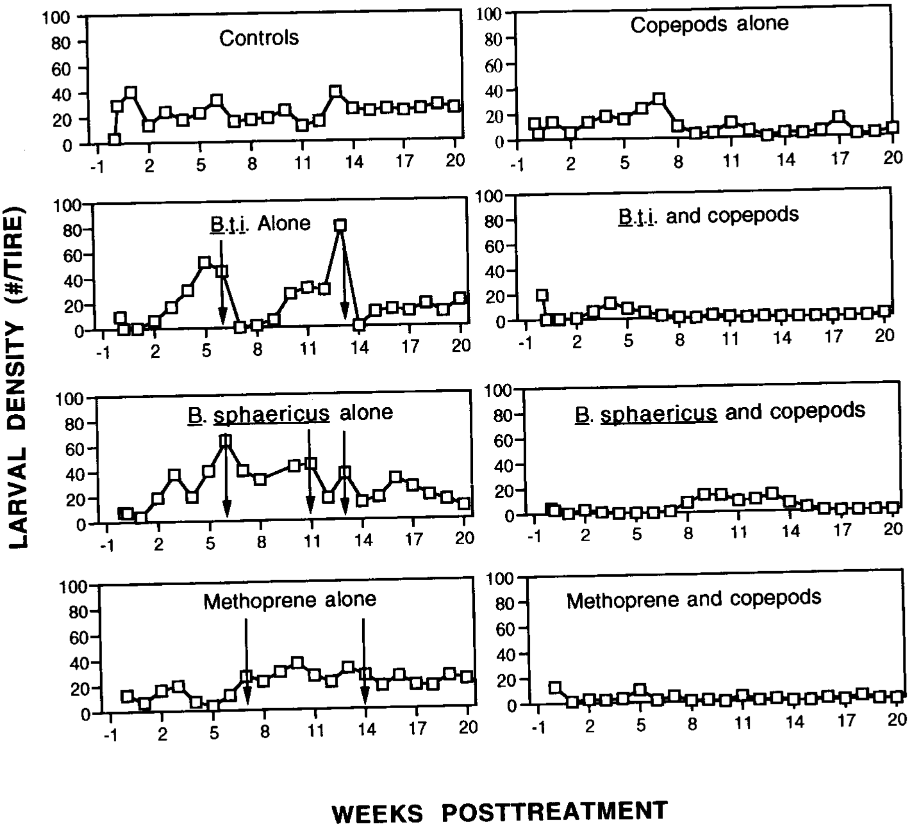

Fig. 3. Mean density of 4th-instar mosquito larvae (all species) per treatment and week posttreatment. Arrows indicate time of reapplication of control agent.

average number of instar/stage *i* during week *j* in the treatment pile. Thus reductions were calculated for: 1) 1st and 2nd, 2) 3rd, 3) 4th instars, and 4) pupae by week posttreatment and type of treatment. Negative percent reduction was reported as zero reduction.

Assessment of the effect of methoprene on pupae collected from tires was different from that of microbials. Pupal mortality assessments were made by collecting pupae from methoprene-treated and control tires and emergence success was determined in the laboratory during the following week to 10 days. Pupae were placed in water in beakers immersed in a temperature-controlled (27°C) water bath. In this way, comparisons were made between numbers of successful emergences versus partially emerged adults and dead pupae. The above frequencies were compiled weekly and compared statistically between samples from piles treated with methoprene, methoprene and copepods, and controls using the *G*-test for heterogeneity (Sokal and Rohlf 1981).

It was necessary to re-treat tires with *B.t.i.*, *B. sphaericus*, and methoprene when no differences were evident between treatment and control populations of mosquitoes as detected by percent reduction for microbial treatments and *G*-test for the methoprene treatments. Treatment concentrations were adjusted to administer the proper dosage based on the water volume of each tire.

RESULTS

Laboratory acute toxicity tests that exposed *M. longisetus* to *B.t.i.* yielded no mortality for treatment or controls at 24-, 48-, or 72-h intervals. Similar tests using *B. sphaericus* resulted in no mortality at 24 and 48 h. For methoprene, a concentration of 197 ppb had no effect on the development of nauplius larvae to copepodids and subsequently to adults. Each of 30 individual copepods exposed to methoprene survived and matured normally, as did the controls. Mating of females collected from treated and control

Table 1. Reduction of immature mosquitoes by instar/stage and weeks posttreatment in tires inoculated with *Mesocyclops longisetus.*

Weeks posttreatment	Reduction (%) by instar/stage			
	L1/L2[1]	3rd	4th	Pupae
0.2	38	65	83	0
1	71	0	66	61
2	65	31	61	95
3	40	0	45	63
4	0	46	0	12
5	28	0	33	28
6	9	0	27	39
7	78	58	0	23
8	96	84	45	62
9	97	96	82	50
10	90	87	83	80
11	62	86	2	0
12	95	72	63	0
13	98	98	97	33
14	99	88	85	92
15	92	89	87	60
16	89	70	81	71
17	96	92	39	0
18	97	99	92	0
19	63	100	91	54
20	0	89	80	97

[1] First- and 2nd-instar larvae.

groups resulted in 50 and 83% gravid, respectively. These results suggest that the microbials *B.t.i.* and *B. sphaericus* and methoprene may be used with copepods without expecting significant deleterious effects.

In the field, pretreatment mosquito populations were predominantly *Culex restuans* Theobald and *Culex salinarius* Coquillett. Both populations quickly diminished during the first 2 months of the study (Fig. 1). Conversely, *Ae. albopictus* was not found in more than 20% of the tires until after water was added by hand 2 wk pretreatment, when a large population increase occurred (Fig. 1). After this time, *Ae. albopictus* occurred in the tires at an average of 52%. Other mosquito species present included *Culex territans* Walker, *Cx. quinquefasciatus,* and the predator, *Tx. r. rutilus.* The Student-Newman-Keuls test indicated that generally, during the first 5 wk of pretreatment monitoring, densities of 3rd- and 4th-instar larvae did not differ significantly ($P > 0.05$) among the 8 piles monitored. During the 2nd wk pretreatment a single pile had a significantly (df = 7; F-value = 2.69; $P = 0.03$) higher density of 4th instars when compared with the other tires. Differences in 1st-instar densities were evident on several occasions, but that was expected due to asynchronous oviposition events from *Culex* spp.

In general, copepod populations sampled during the 1st week postinoculation were found to average 65–79 individuals per tire (Fig. 2). Populations increased sharply during the subsequent 2–6 wk. This was particularly true for the pile "*B.t.i.* and copepods" on the 6th week postinoculation, where the copepods averaged more than 1,200 individuals per tire (Fig. 2). During 14 weeks of the 20-wk posttreatment interval, no significant differences (student's t-test; $P > 0.05$) were detected in copepod density among the 4 inoculated tire piles. The tire pile "*B.t.i.* and copepods" had significantly higher (student's t-test; $P < 0.05$) copepod populations than other piles during 2, 5, and 11 wk postinoculation (Fig. 2). Although most tires contained greater than 100 copepods by the 2nd or 3rd week to the end of the study, the population in one tire of the "copepods alone" pile slowly declined from 47 to 0 during the first 16 wk postinoculation. The latter tire was not excluded from analyses. During the 12th week postinoculation a tire in the "methoprene alone" pile became infested with copepods. After repeated attempts to remove them, it became necessary to replace that tire to maintain adequate replication.

Immature mosquito density in tires inoculated with copepods alone was high during the first 7 weeks posttreatment, but subsequently decreased and remained low to the end of the study (Fig. 3). High mosquito densities were consistently found in the tire where the copepod population had never increased beyond 47 individuals. This tire greatly influenced the averaged mosquito density in this treatment. Overall, reductions of 3rd instar mosquito larvae ranged from 0 to 98.8%, averaging about 64% during the entire 20 week interval (Table 1). Similarly, reduction of 4th instar larvae averaged 58% when exposed to copepods alone (Table 1).

Tires treated with *B.t.i.* alone had cyclic mosquito abundances that reflected short-term effects of this microbial on their populations (Fig. 3). *Bacillus thuringiensis* var. *israelensis* was found to be effective for up to about 2–3 wk after each application, but in each case, reapplication was necessary to continue an adequate degree of supression. Replications were made 6 and 13 wk posttreatment to create a *B.t.i.* concentration of 1,565 ppb. In contrast, tires containing copepods that were treated with *B.t.i.* produced only minimal numbers of immature mosquitoes during 3–5 wk posttreatment (Table 2). Reapplications of *B.t.i.* to these tires were deemed unnecessary.

No prolonged depression of mosquito density was detected in tires treated with *B. sphaericus*

Table 2. Reduction of immature mosquitoes by instar/stage and weeks posttreatment in tires treated with *Bacillus thuringiensis* var. *israelensis* with and without the copepod *Mesocyclops longisetus* present.

	Reduction (%) by instar/stage							
Weeks post-treatment	Without copepods				With copepods			
	L1/L2[1]	3rd	4th	Pupae	L1/L2	3rd	4th	Pupae
0.3	100	100	100	100	99	100	100	0
1	97	98	100	100	76	100	100	100
2	97	91	56	100	77	97	94	100
3	62	0	30	74	26	89	74	48
4	0	0	0	0	68	86	28	37
5	9	0	0	0	92	46	65	54
6	0	0	0	0	100	57	85	54
7	74	99	100	100	100	97	86	95
8	49	93	91	94	96	100	97	100
9	65	80	69	83	75	100	100	100
10	67	33	0	0	98	99	91	90
11	55	0	0	0	100	100	94	83
12	0	0	0	0	100	99	100	100
13	24	0	0	0	100	100	100	100
14	91	100	100	100	100	100	100	100
15	69	45	50	70	100	100	100	100
16	66	29	47	0	100	100	100	100
17	96	88	49	0	100	100	100	100
18	86	76	30	50	96	100	100	100
19	63	54	61	0	85	98	100	100
20	0	72	20	3	0	99	93	100

[1] First- and 2nd-instar larvae.

alone, in spite of repeated attempts at increasing concentrations: 1,584 ppb (1.2 liter/ha), 3,168 (2.3 liter/ha), and 6,336 ppb (4.6 liter/ha) on 6, 11, and 13 wk posttreatment, respectively (Fig. 3 and Table 3). Where *B. sphaericus* treatments were combined with copepods, mosquito numbers were suppressed during most of the study, although a slight resurgence was noted from 8 to 14 wk posttreatment (Table 3). Overall reductions of 3rd and 4th instars in the *B. sphaericus* pile averaged 41 and 19%, respectively, whereas *B. sphaericus* and copepods averaged 82 and 79%, respectively.

Larval mosquito populations appeared largely unaffected in tires treated with methoprene alone as would be expected for an insect growth regulator (Fig. 3). Methoprene reapplications (19.7 ppb) were made 7 and 14 wk posttreatment based on nonsignificant ($P > 0.05$) pupal mortality when comparing treatment and control tires. In comparison, minimal numbers of juvenile mosquitoes were collected in tires where methoprene and copepods were integrated (Fig. 3). Further applications of methoprene were not necessary in copepod-inoculated tires. Methoprene and copepods resulted in an average of 95% reduction of 3rd- and 90% reduction of 4th-instar larvae (Table 4). Although reduction of 3rd and 4th instars in the methoprene tire pile averaged 21% each, pupal mortality was significant ($P < 0.05$) during much of the posttreatment interval (Fig. 4). Pupal mortality generally declined after each application, but remained significantly greater ($P < 0.05$) than that in control tires ranging from 4 to 7 wk postapplication, suggesting a prolonged residual activity for methoprene in tires.

Overall densities of immature mosquitoes varied significantly among tire pile treatments based on general linear model analyses (Table 5). Significantly lower densities of 3rd and 4th instars were consistently found where copepods were integrated with *B.t.i.*, *B. sphaericus*, or methoprene. Copepods alone also yielded significantly lower densities of 3rd- and 4th-instar larvae and pupae when compared to controls (Table 5). Overall, numbers of 4th-instar larvae were not significantly different between controls, *B.t.i.*, *B. sphaericus*, and methoprene treatments. The latter observation was due to the transitory effect of those treatments, whereas copepod-integrated piles had more uniform results.

Water temperature minimums in waste tires

Table 3. Reduction of immature mosquitoes by instar/stage and weeks posttreatment in tires treated with *Bacillus sphaericus* with and without the copepod *Mesocyclops longisetus* present.

Weeks post-treatment	Reduction (%) by instar/stage							
	Without copepods				With copepods			
	L1/L2[1]	3rd	4th	Pupae	L1/L2	3rd	4th	Pupae
0.3	93	81	75	0	92	97	91	0
1	69	85	90	61	94	90	99	96
2	39	62	0	30	98	91	80	70
3	26	0	0	0	96	93	96	89
4	0	43	0	0	94	99	100	81
5	19	0	0	0	100	100	100	96
6	0	0	0	0	98	90	100	96
7	43	0	0	0	100	99	95	90
8	20	53	0	0	51	49	55	100
9	—	—	—	—	36	48	26	0
10	2	68	0	0	71	52	44	10
11	0	17	0	0	80	13	27	0
12	34	0	0	0	93	52	37	67
13	23	25	4	0	97	83	65	44
14	72	88	42	0	99	90	73	58
15	0	30	24	0	100	95	86	100
16	6	33	0	0	100	100	98	100
17	56	65	0	0	100	100	100	90
18	0	63	22	0	100	100	100	100
19	5	56	45	0	98	100	100	100
20	0	37	59	59	55	100	100	100

[1] First- and 2nd-instar larvae.

Table 4. Reduction of immature mosquitoes by instar/stage and weeks posttreatment in tires treated with methoprene with and without the copepod *Mesocyclops longisetus* present.

Weeks post-treatment	Reduction (%) by instar/stage							
	Without copepods				With copopods			
	L1/L2[1]	3rd	4th	Pupae	L1/L2	3rd	4th	Pupae
1	63	0	83	90	94	85	97	96
2	71	32	0	76	87	82	80	86
3	48	0	19	48	80	96	91	67
4	47	77	60	75	85	89	81	100
5	66	47	83	68	99	91	57	89
6	41	0	64	54	90	98	95	96
7	19	35	0	48	100	94	71	86
8	0	13	0	62	100	99	96	94
9	14	0	0	83	99	99	93	58
10	18	0	0	10	100	80	100	100
11	3	0	0	17	100	90	67	83
12	22	0	0	17	96	99	97	100
13	46	25	17	11	100	99	96	78
14	28	43	0	0	100	100	100	100
15	15	20	24	70	98	100	100	100
16	43	26	0	36	100	100	94	100
17	5	32	23	60	97	100	100	100
18	0	18	30	67	100	100	88	100
19	8	0	12	46	100	99	99	92
20	0	54	10	86	67	100	100	96

[1] First- and 2nd-instar larvae.

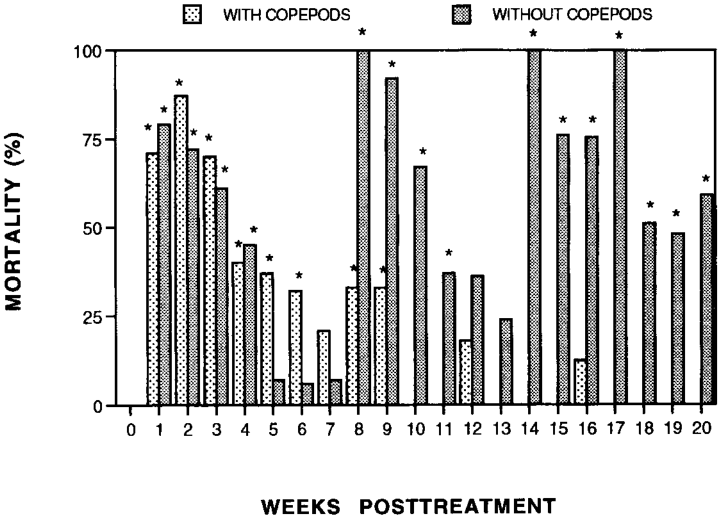

Fig. 4. Pupal mortality in waste tire piles treated with methoprene in treatments with and without copepods by week posttreatment. Asterisks denote significant differences ($P < 0.05$) compared to control pupal mortality using the *G*-test.

ranged from 7.25 to 25°C, and maximums ranged from 24.4 to 34°C.

DISCUSSION

Laboratory bioassays indicated that *Mesocyclops longisetus* tolerate methoprene at 0.197 ppm. These results were comparable to those of Marten et al. (1993) who reported survival of *Macrocyclops albidus* in methoprene concentrations less than 0.21 ppm. They also found *M. albidus* to be unaffected by *B.t.i.* Bircher and Ruber (1988) found the salt marsh copepod *Apocyclops spartinus* to be susceptible to methoprene when exposed during the early nauplius stage (LC_{50} = 0.8 ppm). This value, however, greatly exceeds our predicted field concentration.

During the posttreatment interval, immature mosquito density fluctuated widely in untreated waste tires. These changes were greatest for early instar larvae, which ranged from 0 to 500 individuals per tire during the 20-wk posttreatment interval. These changes may be attributed to periodic flooding episodes that caused recruitment or *Ae. albopictus* egg hatches. In contrast to the larval stage, pupae were more consistent in density.

Reduction of immature mosquitoes in *B.t.i.* (only) tires reflected the temporary effect of each application (Table 2). Each treatment of *B.t.i.* into these tires yielded close to 100% reduction for 2 wk posttreatment; after the 3rd application, moderate reduction continued several weeks longer. In the *B.t.i.* and copepods treatment a similar initial high degree of reduction was displayed due to the microbial (Table 2). It is apparent that, shortly after the efficacy of the first *B.t.i.* treatment waned, copepod-induced reductions rose to above 90%. Copepod populations in *B.t.i.* treatments exceeded 100 per tire by the 3rd week, when the microbial effects declined. During weeks 3–7, a high degree of reduction of 1st- and 2nd-instar larvae preceded that of later instars, thus demonstrating how early elimination of the developing mosquitoes almost completely precluded the presence of later stages (Table 2). These results are in accord with Marten et al. (1993) where *B.t.i.* was combined with *M. albidus* in a 30-day field study.

The toxicity of *B. sphaericus* varies widely when comparing sensitivities of larval *Aedes* and *Culex*, where the latter genus is susceptible and the former is more tolerant (Silapanuntakul et al. 1983, Gardner et al. 1986, Mulligan et al. 1978). The utility of *B. sphaericus* in tire mosquito control programs will largely depend upon whether or not *Aedes* is the predominant genus present.

This study determined that copepods are compatible with *B.t.i.*, *B. sphaericus*, and methoprene at concentrations exceeding those labeled for operational mosquito control. A 5-month field test, integrating the copepod *M. longisetus* with either *B.t.i.*, *B. sphaericus*, or methoprene provided better mosquito reduction than either copepods or control agents utilized alone. When copepods were combined with *B.t.i.* or methoprene, reduction of 3rd and 4th instars was equal to or greater than 90%. *Bacillus thuringiensis* var. *is-*

Table 5. Mean number ± SE of immature mosquitoes per tire during 20-wk posttreatment interval by waste tire pile treatment. Unshared letters among waste tire pile treatments denote significant differences ($P < 0.05$) based on Student-Newman-Keuls multiple range tests.

Mosquito instar/stage	Waste tire pile treatment							
	Control	Copepods	*B.t.i.*	*B.t.i.* and copepods	*B. sphaericus*	*B. sphaericus* and copepods	Methoprene	Methoprene and copepods
First/second	208 ± 16a	67 ± 14cd	80 ± 11c	38 ± 10de	155 ± 12d	27 ± 11e	143 ± 12b	14 ± 4e
Third	25 ± 3a	12 ± 3c	15 ± 2bc	2 ± 1d	15 ± 2bc	4 ± 2d	20 ± 2ab	2 ± 0d
Fourth	23 ± 2ab	10 ± 2c	19 ± 3b	3 ± 1d	26 ± 3a	4 ± 1d	20 ± 2b	2 ± 1d
Pupae	5 ± 1b	3 ± 1c	6 ± 1b	1 ± 0c	8 ± 1a	2 ± 0c	2 ± 0c	1 ± 0c

raelensis alone was found to temporarily produce a high degree of larval reduction; however, reapplications were necessary to maintain that level of control. Methoprene may have had longer lasting effects than *B.t.i.* but also required reapplication. Of all the treatments, *B. sphaericus* alone produced the lowest degree of mosquito suppression due to low level of toxicity to *Ae. albopictus,* the predominant species during the study. It is recommended that to optimize *Mesocyclops* survival and population growth as was evidenced in this study, the timing of the releases (in the southeastern United States) should be targeted for spring (April–May) and the initial inoculation rate should not be less than 100 copepods per tire (Schreiber et al. 1993). It is recommended that mosquito control operators implement integrated pest management against mosquitoes in waste tires using *M. longisetus* and *B.t.i.* or methoprene. Because *M. longisetus* is indigenous to the southern United States (Marten 1990a) permits should not be required for field releases.

ACKNOWLEDGMENTS

We thank the following people for their participation in this project: James Clauson, Edward Hunter, Charles Hallmon, Mike Gaffney, and those involved in reviewing this manuscript. The New Orleans Mosquito Control Board is acknowledged for originally providing copepod stocks used in this study. Funds for this study were provided by the Florida Department of Agriculture and Consumer Services contract #1417.

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